# Selection of bioactive terpenes and reproductive cycles of *Cannabis sativa, C. indica*

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## EXECUTIVE SUMMARY.

The objective of this review is to get back to the roots of the story so that future endeavors in manipulating the genetics/production tactics cannabis can be a lasting success. This paper will primarily review the history and future production opportunities of *Cannabis saliva L.* and *C. indica L.*, although it will also cover the breeding potential for auto-flowering/ short season plants by crossing with *C. ruduralis*. Developing methods to grow cannabis for constant harvest, perennially and disease resistance could significantly reduce production costs and increase efficiency.

# I. INTRODUCTION

## A. Study Species.

### A Brief History of *Cannabis sativa, C. indica* production, uses and legality

Cannabis production in the US is a rapidly expanding market; greenhouse growers across the country are being carefully selected to provide medicinal grade marijuana to the healthcare industry. This renewed interest in the therapeutic application of cannabis has led to innumerable new products and growth systems all aiming to maximize yields and quality. In fact, the legal marijuana industry grew by 74% from 2013 to 2014 according to ArcView Group, a marijuana market research and investment firm based in San Francisco, CA (Dayton 2015). The industry made $2.7 billion in revenue during 2014, which is up from $1.5 billion in 2013; ArcView group estimates the national legal marijuana market will reach $10.2 billion by 2019 (Dayton 2015). This is a rapidly growing and highly profitable industry, and the scientific, horticultural community at large could benefit from a greater understanding of the species to help shape the future industry in terms of production methodology and genetic breeding. Though, in a modern sense, it is a relatively new product market with its recent legalization status in various states, the plant species within the *Cannabis* genus have been part of the human experience since prehistoric times. *Cannabis sativa* is known to have been harvested by the Chinese at least 8500 years ago for paper and textile making (Wang et al. 2009). The morphology and legality issue that surround the female plant (marijuana) are what comes to mind when most people think of *Cannabis*. However, for the majority of its historical use by man, it has been primarily valued for its fibers and less so for intoxicants or oil (Wang et al. 2009). Male cannabis plants (hemp) may be used in a wide variety of ways; its strong, durable fibers from the stalks can be used to create textiles, cords and fiberboards. Hemp and marijuana differ primarily due to the reasons behind cultivation; hemp is grown for its fibers and seeds while marijuana is grown for its buds that often contain high levels of cannabinoids such as Tetrahydrocannabinol (THC) and Cannabidiol (CBD).  Cannabinoids are compounds in cannabis that give the flower buds their medicinal properties. Since the human brain is equipped with cannabinoid receptors it is possible to directly affect the central nervous system using marijuana flowers. There are over 60 types of cannabinoids present in cannabis; the two highest in concentration and, thus, the most studied, are THC and CBD (Joy et al, 1999).

This species has been going through a significant transition period in recent times as its legality switches from one end of the spectrum to another. Interestingly, in 1619 the first colonist settlers of the United States of America were legally bound to grow cannabis for the government since it's strong fibers were so valuable in producing wartime supplies (Deitch, 2003). It wasn't until the 1930s, with the introduction of the Uniform State Narcotic act (1925-1932), the Federal Bureau of Narcotics (1930) and the Marijuana Tax Act (1937) that the US federal government categorized *C. sativa* and *indica* officially as Schedule 1 drugs in the Controlled Substances Act of 1970 (PBS, 2014). Recently these laws have begun to be overturned at the state level; recreational sale and possession became legal in Colorado and Washington on November 6, 2012, and since then an ever-increasing number of states (28+) have been following suit. Policies are passed through state governments and determine varying degrees of autonomy and restrictions in regards to production, distribution and possession. The State of Minnesota 's marijuana law was passed and signed June 2014, and, though it allows for only very specific medicinal applications of cannabis derivatives, it has nonetheless opened the doors for in-state production (PBS, 2014).

Commercial production of cannabis is a booming industry in many areas, as more states legalize medical cannabis use the opportunity for growth expands in this multibillion-dollar industry (Dayton, 2015). Highly regulated greenhouses with state of the art technology and genetics are at the forefront of the revolution (Bayley, 2014). There is demand for development of both production tactics and breeding projects, extensive research and experimentation has gone into optimizing yields, adapting plants to various growth habitats and fine-tuning terpene/cannabinoid expression (Dayton, 2015; Fishedick et al., 2010; Vanhove, 2011; William, 2007). Specifically, manipulating THC and CBD concentrations in the flower buds opens the door for both precise medicinal application and industrial hemp production for growing markets (Fischedick et al., 2015).

In order to address the obstacles faced with current methodology and create the next wave of game-changing plants, breeders and growers alike must have a thorough understanding of not only the newest advancements in the field, but also the complex biological history of the plant itself. The objective of this review is to get back to the roots of the story so that future endeavors in manipulating the genetics/production tactics of the species can be a lasting success. This paper will primarily review the history and future production opportunities of *Cannabis saliva L.* and *C. indica L.*, although it will also cover the breeding potential for auto-flowering/ short season plants by crossing with *C. ruduralis*. Developing methods to grow cannabis for constant harvest, perennially and disease resistance could significantly reduce production costs and increase efficiency.

## The Biological History of Cannabis

Cannabaceae are a group of land plants that contain vascular structures and are Angiosperms, meaning they produce seeds in a closed carpel (Hillig 2005). Their leaves are branching and veined and serrated with alternating leaves with stipules around the stem and 5 petals/tepals within the flowers (Hillig 2005).

## Growth Habits

As previously mentioned, all *Cannabis* species are in the hemp family, otherwise known as Cannabaceae. Physically, this implies they have separate male and female plants, are wind pollinated and often have radial symmetry in their leaf clusters (Williams 2007). The female plant contains the genetics to form ovaries in the form of flower buds and the male plant produces pollen to fertilize the flowers. Only upon pollination will the female plant produce seeds, generally this is done for the plant by the wind, which carries the pollen from the anthers of a male to the stigma of the female flower. Cannabis plants grow as annual dicots, meaning the embryo contains two cotyledons or seed leaves, and are well adapted to a variety of environments, though they prefer humid, warm sandy loam soil with a pH between 5-7 (William 2007). They are vascular plants that lack significant woody tissue above the ground and have one major taproot with numerous peripheral roots that spread out from the center to form a dense network. They are able to convert nutrients fairly quickly, as seen in their rapid growth and ability to grow to staggering heights in just one season (William 2007). Typically cannabis blooms June-September and will grow anywhere from 20-100cm (WSH 2015). Though not native to North America, *Cannabis sativa* is incredibly adaptable and can thrive in nearly any environment.
If producers are growing outdoors there is a fair chance that the plot in which the crop is being grown will continue to sprout up seedlings annually, as seen with corn fields in Iowa that were

converted to hemp production during World War II (William 2007). It can be found in nearly every state in it's naturalized form (besides Nevada), as seen in Figure 2 (NRCS, USDA 2015). Though cannabis can be grown in Nevada there is no documentation of it naturalizing as weed in that landscape, this could be due to the desert qualities of the environment which are unfavorable to it's growth.

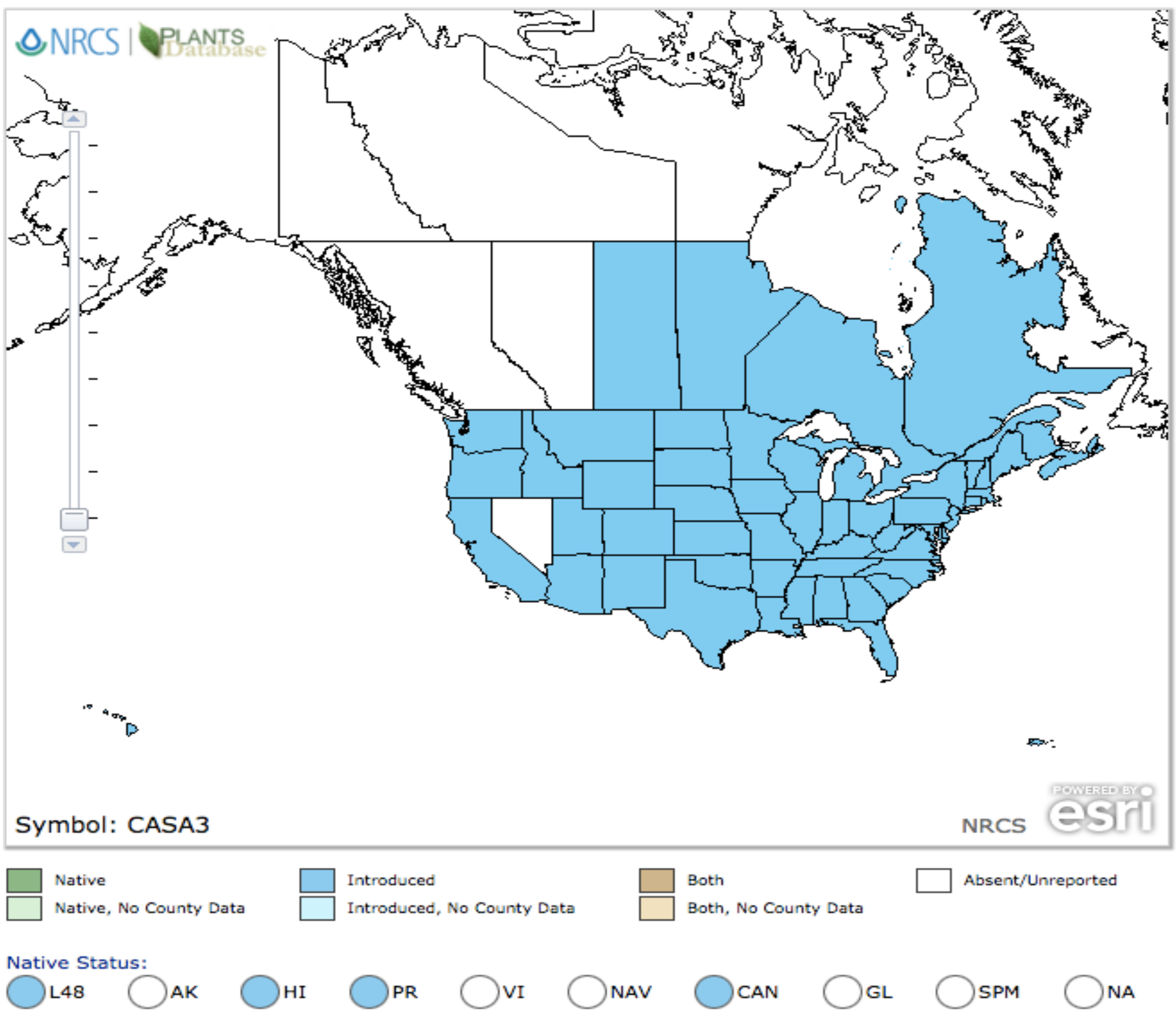


Figure 1. Locations where *Cannabis* currently grows as an introduced weed in its naturalized form (Adapted from the NRCS, USDA. 2015).

**Native Habitat**

Since cannabis is native to southern/ central Asia, it has a preference for humid, warm climates with lots of sunlight (Williams 2007). *Sativa* and *indica* varieties are long-day obligates in terms of their photoperiodism, meaning they require at least 12 hours of light to begin flower initiation (Williams 2007). Cannabis thrive in full light and will produce the highest yields and concentrations during 18 hour days, though the exact light and maturation rates will vary based on the species and cultivars grown (Williams 2007). In the wild, *Cannabis* can grow from seed to harvest in about 8 months, whereas indoor production standards complete the same cycle in just 90 days (Williams 2007).
The map below (Figure 4) shows the spread of *Cannabis* in chronological order. Over the centuries the species has been able to spread and survive in nearly every corner of the world, though it was only within the past 200 years that it was brought to the Americas.

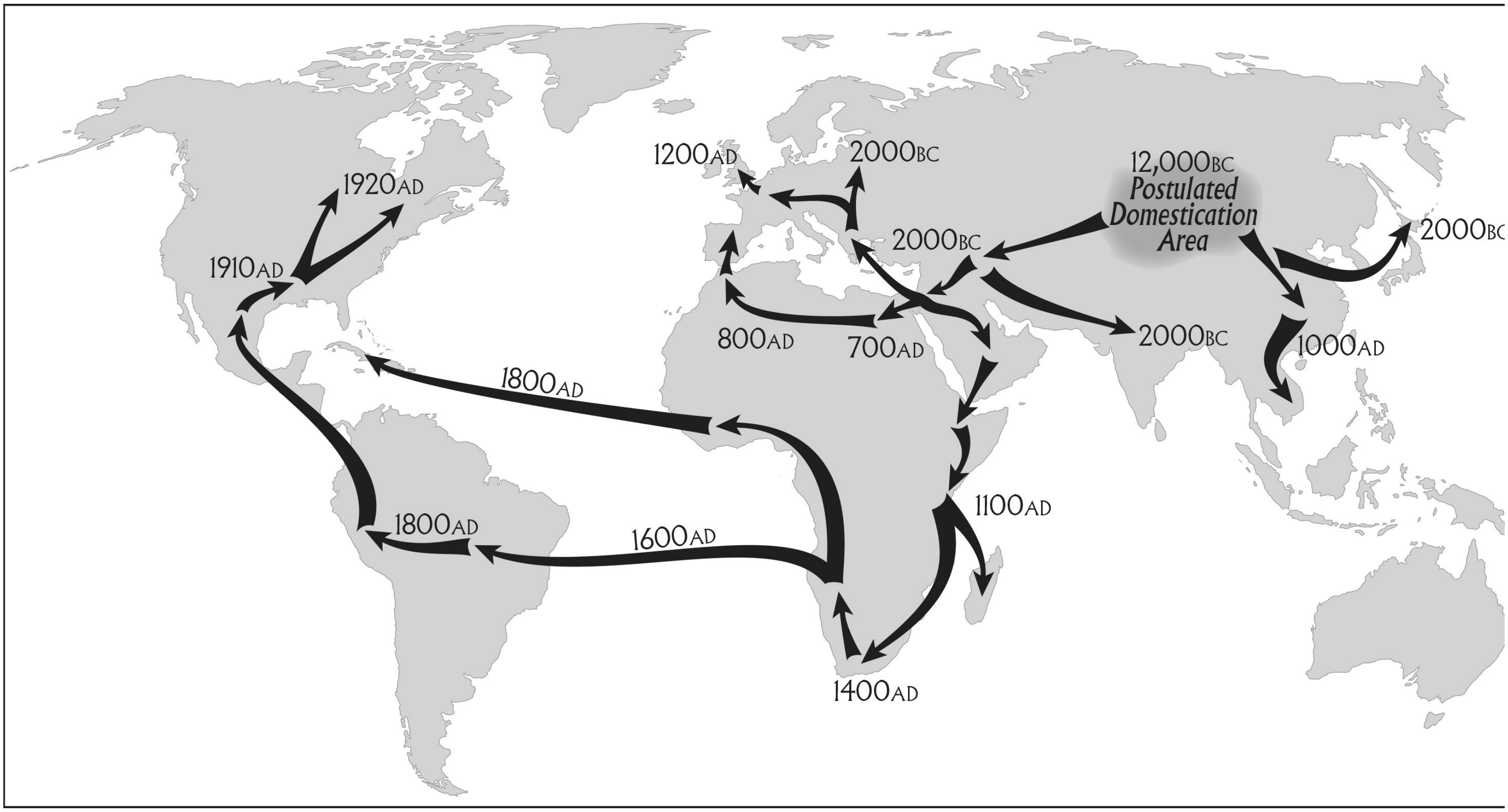


Figure 2. Map showing the historic spread and distribution of marijuana throughout the globe (Warf B. 2014).

When grown indoors, supplemental lighting is used to maximize vegetative growth and replicate necessary photoperiods to initiate flowering. High intensity lighting is used (up to 1000W) to produce maximum yield. Temperatures are kept optimally between 24-30°C and require extensive ventilation systems to remove excess heat from the high intensity discharge (HID) lights. Grow rooms must also keep close control over air quality to ensure proper levels for the plants and to minimize disease. The atmosphere needs to be kept at precise humidity levels and may be supplemented with $CO_2$ in varying amounts depending on the plants maturity (Williams 2007). Cannabis can be grown successfully in many environments, yet it takes refinement of technique and thorough knowledge of production to maximize yield and create quality product. From field soils to indoor potting mixes, soilless media types in hydroponics, aquaponics, and vertical growth structures this plant will grow as long as the pH, nutrient requirements and general environmental needs are being met.

**B. Taxonomic Classification and Geographic Distribution in the Wild.**

*C. indica and C. sativa* are generally accepted as the two main species of *Cannabis*, though there is a third species not used commercially: *C. ruderalis* J. Each species is distinct in its appearance (height, stature, inter-nodal length, leaf structures), bud sizes/densities, flowering times and terpene/ cannabinoid expression (Gloss, 2015). Terpenes are pungent oils produced by plants that give varieties their specific flavors and scents. Many plants produce terpenes for survival since they can both attract pollinators and deter predators. Terpenes have an important role in the way the body metabolizes both cannabinoids and naturally produced neurotransmitters such as dopamine and serotonin (Rahn 2014). Each species of *Cannabis* has its' own specific blend of bioactive chemicals expressed through the trichomes on their flower buds, more detail on the importance and function of terpenes and cannabinoids will be explained later. For now it is important to keep in mind that the driving factor behind production of marijuana is the quality and quantity of trichomes obtained at the final stages of harvest and curing.

As mentioned previously, the three recognized species of Cannabis are *indica, sativa* and *ruderalis.* Though most cannabis plants grown as medical marijuana are *Cannabis sativa x C. indica* hybrids, besides a select few landraces that have been intentionally preserved through the years. Below is a summary of each species and its' unique physiological and biochemical attributes.

*Cannabis indica* is also commonly referred to as hashish or Indian Cannabis since it is believed to have originated in the Hindu-kush region of Central Asia. This species was brought to the US during the 1970s from Afghanistan and allowed for the very first hybrids to be created (Gloss 2015). They are shorter, bushier plants with broad leaves and can be found growing naturally in mountainous regions with sandy loam soils. Indica strains typically have a high THC:CBD ratio (Fischedick et al. 2015) which leads to a more body- heavy and physical high useful for night time use.

*Cannabis sativa* is likely the best known species and may also be called marijuana or European hemp. There are native species to both Central America and the equatorial tropics of South America which grow very tall (2-4m) and have thin, long leaves (Gloss 2015). The buds induce a vivid, cerebral high, which may inspire creativity/ action, or produce anxiety in some people. This is because *Sativa's* generally have a higher CBD:THC ratio and target specific neural pathways in the brain(Fischedick et al. 2015).

*Cannabis ruderalis* is the least known species, generally because it is not used in production due to its small yields and lack of vigor. However, they are genetically valuable due to their autoflowering nature (flowering due to age instead of light conditions), cold hardiness and rapid maturity rate (Gloss 2015). *C. ruderalis* is indigenous to Eastern Europe/ Russia and is adapted to cool climates and short days, this plant can go through its entire life cycle in just 10 weeks and it's seeds can survive the frigid Siberian winters (Gloss 2015). This species could potentially provide valuable production traits to current cultivars if proper breeding programs are developed to hybridize them.

**Reproductive cycle and properties of interest**

Cannabis plants have two sexes but can occasionally be hermaphrodites if they possess both sets of organs (Williams 2007). The male plants produce pollen and contain the anthers, which rest on filamentous stalks. Female plants produce seeds and have a sticky stigma that rests at the tip of a style that catches wind-borne pollen (Williams 2007). From there a pistil transports pollen to the ovaries which house the future seeds and cannabinoid-rich 'buds', though ovules within the ovary become seeds if fertilized (Williams 2007).

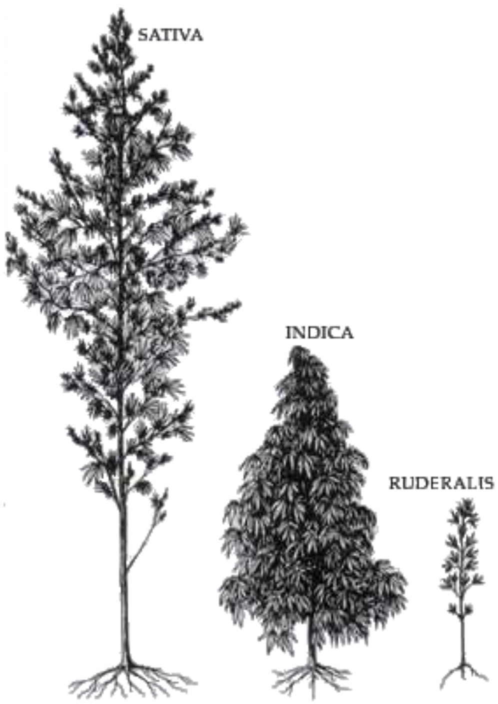


Figure 3. Different statures displayed by the main *Cannabis* types (Wikipedia, 2008)

Both *indica* and *sativa* species require 12 hours of light and 12 hours of darkness to begin flower bud initiation after they reach adequate vegetative growth size.*C. sativa* grows best in intensive light and will produce the highest yields during 18-20 hour days, however producing a pure sativa strain is generally not as economically viable due to its extended maturation time and expensive light requirements. By creating interspecies hybrids it is possible to utilize the best qualities from each gene pool to create well-adapted plants with a plethora of cultivars to meet every need.

There is a good deal of interest in high CBD, low THC strains in the medical world at this time since they can be used as pain relief without the psychoactive properties and may be helpful in addressing cancer, epilepsy, inflammatory conditions, nausea, and anxiety/ depression (Schwartz 1997). Having a scientific understanding of the biochemical interactions that occur between the plant and human body reveals the true value of growing *Cannabis,* namely the terpene and cannabinoid expression found in the trichomes produced by the flower buds. The role of terpenes is similar to that of a catalyst in many chemical reactions, these substances dictate the part of the brain that is activated by the cannabinoids present in the plant material. Some terpenes are particularly helpful in lifting the mood and relieving stress, while others have more of a focusing effect.

Terpenes within the plant act subtly but can make a big difference in the way a strain interacts with and individuals' body chemistry; they add another layer to the complex cocktail of bioactive compounds present in marijuana. Both terpenes and cannabinoids directly bind to both brain (CB-1) and body (CB-2) receptors and can have a variety of effects based on what type they are and where they bind (Rahn 2014). These compounds replicate endocannabinoids- hormone substances naturally produced by the body and actively involved in maintaining internal stability and wellbeing. Endocannabinoids act as communicators between cells and are involved with transmission of signals such as nausea, pain, mood and general nervous system operation (Rahn, 2014). Controlling the expression of cannabinoids and associated terpenes in cannabis has such widespread therapeutic use because it replicates the bodies natural system of pain management. THC tends to bind to the CB-1 receptors and acts as a partial agonist in the brain, meaning it can help uplift the mood and create more of a vivid, cerebral "high" (Rahn, 2014). While CDB, which tends to bind to CB-2 receptors in the body, acts as both a serotonin receptor agonist and a cannabinoid receptor antagonist that can be used to minimize the effects of physical pain while retaining a clear mind (Joy et al. 1999). There are numerous other cannabinoids with specific physiological functions, though THC and more recently, CBD are the two of most interest at this time. Even for people who do not smoke, pain relieving salves, lotions and pills can be made from the extracts of these potent plants.

*Cannabis* seeds are also quite valuable since they have a very high energy density and  are a nutritionally complete protein- they provide all the essential amino acids necessary in the diet. Seeds can be used as nutritious foods, flours, fuel alternatives and as ingredients in the natural health/body care industry. Figure 5 (below) helps lay out the various cannabinoid applications for different disorders within the body and can be used as a resource when choosing the cultivars intended end function (Rahn 2014).

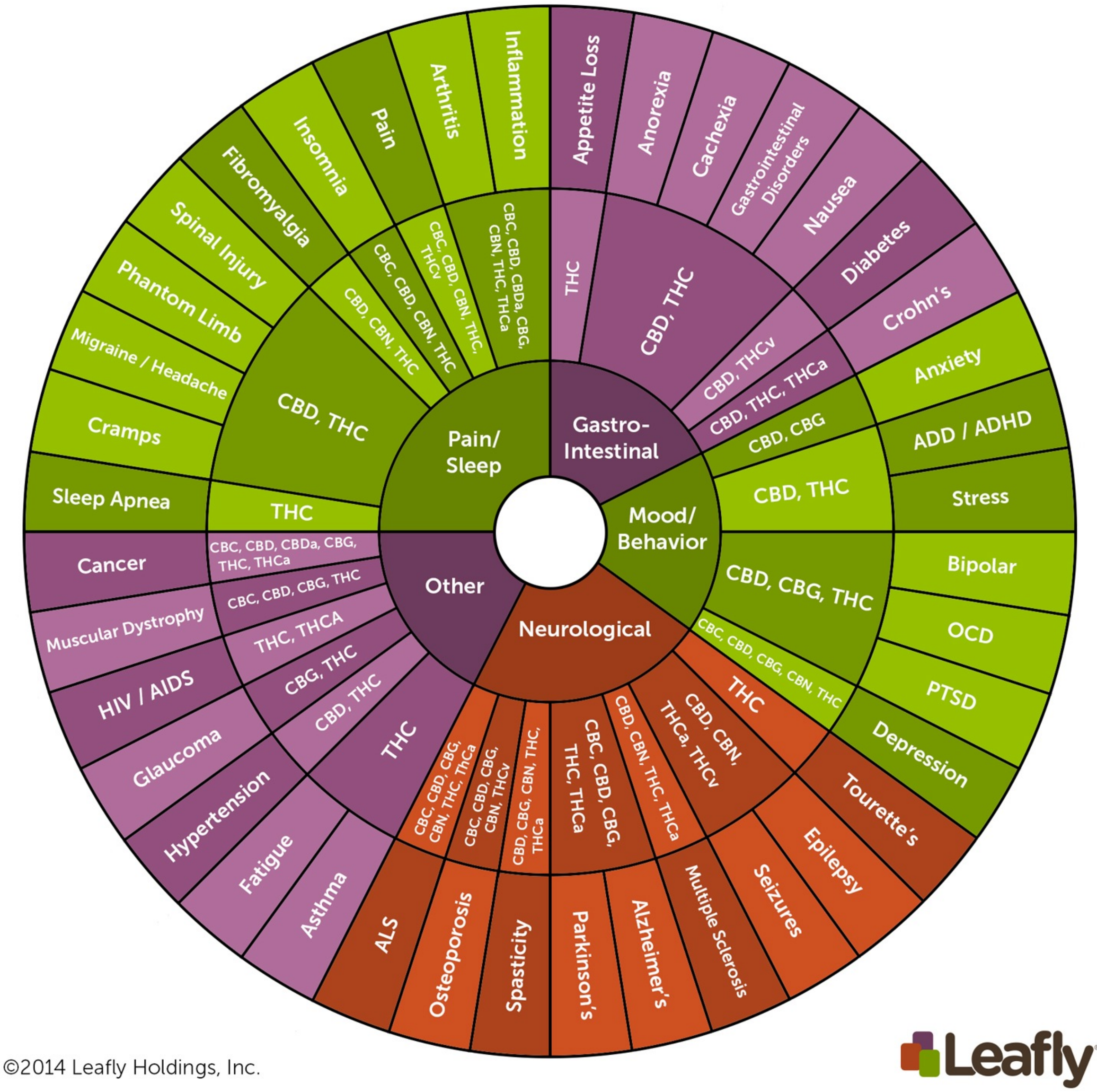


Figure 4. Diagram of the various medical applications of various bioactive terpenes present in *Cannabis.* (Adapted from Leafly Holdings, Inc. Rahn, 2014)

## II. CROP HISTORY

### A. Breeding & Domestication.

Since cannabis has been part of human history for so long, and due to the restrictions on scientific investigation due to drug policy, the evolutionary and domestication history of *Cannabis* remains a bit of a mystery (Sawler 2015). Originating in Asia and spreading throughout the globe for countless generations, farmers most likely would select the best seeds from the wild and then the best seeds from their own crops annually. This gave rise to purebred landraces or ecotypes, or plants that have existed in a particular area for so long they all express similarities in their genetics (Sawler 2015). During the 1960s when worldwide travel, breeding technology and interest in cannabis production were all on the rise, exotic landrace *sativas* were brought to the US and produced in California (Bayley 2014). It wasn't until the 1970s that the first *indica* strains were introduced, and by the 1980s subspecies crosses were on the rise and created the many hybrids used in breeding today (Bayley 2014).

Currently there are a number of both private and public breeding programs working on altering cannabinoid expression, growth rates, photoperiods, and resistance/hardiness for both medicinal and industrial cannabis. Once breeders release their finished seeds (or clones) for sale, the next step is the producer or farmer who grows the plants up for specific end products. The farmer may be their own distributor or could supply wholesale product to specialty processors further down the line. Generally, the grower is responsible for harvesting and curing the plant products before moving to the next stage. Once the product reaches the retail level a number of things may be done to it: buds can either be sold as dry weight, or processed into extracts, concentrations, and edibles for medicinal or recreational use. Hemp grown for seed can also be sold as dry weight, powder, and oil while fiber hemp can be processed into fabrics or fiberboard. Depending on customer needs and preferences they may choose to purchase the processed products, dry weight buds, seeds or clones directly from breeders. The diagram below shows the horticultural distribution chain of *Cannabis.*

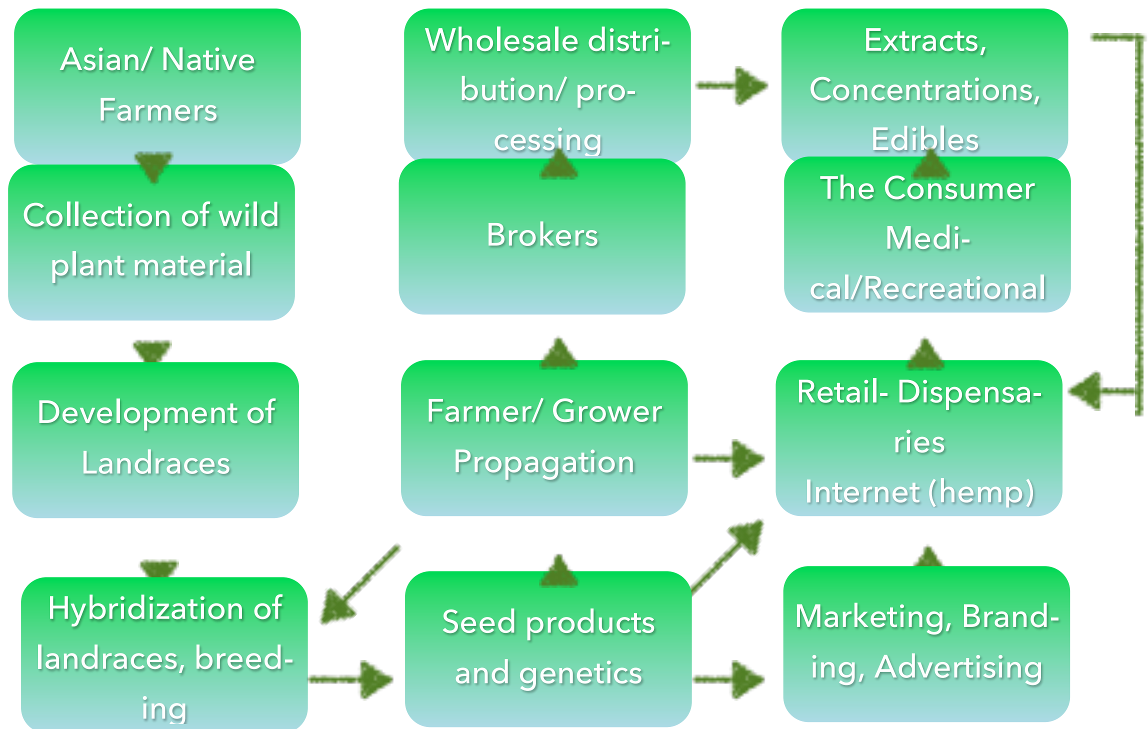


Figure 5 outlines the horticultural distribution chain for *C. sativa/ indica* (Schwier, 2015).

There is an ongoing investigation in understanding the genetic makeup of the hundreds of cultivars on the market in hopes of continually refining and enhancing production and quality. Many research teams worldwide have been analyzing the various chemotaxonomic terpenoid variations of cannabis in order to more closely standardize their use as medicine (Fischedick et al. 2015). In fact, the complete 400 million base-pair genomic sequence of *Cannabis sativa* was published online by Medical Genomics and Nimbus Informatics in 2011, at the same time Canadian researchers reported sequencing the draft genome of *Cannabis indica* ,'Purple Kush'  (Van Bakel 2011). Understanding the genetic makeup of cannabis allows breeders to select for qualities that lend themselves to both highly controlled, scheduled greenhouse production and the chemical pallet of the consumers (terpene expression and potencies).

Figure 6 is an example of the horticultural distribution chain of *Cannabis*. It shows how wild plant materials are continually refined and processed the a number of routes it may take to meet specific needs of the end consumer. Though the diagram represents the physical movement of the product through the industry, it does not accurately represent the political influence that dictates quantity, customer and growing trends. Only established greenhouse growers are allowed to produce medical marijuana products in many states and the chain in these operations is very tightly regulated. In states that allow recreational sale of Cannabis, growers and customers alike have much more flexibility in regards to genetics, production methods and final product processing. Each system has it's own unique guidelines which should be thoroughly understood prior to undertaking any type of production. Being familiar with both state and federal regulations is important to any job, can be complex when producing and distributing Cannabis since the legality varies so widely from place to place.

## A. Current Production Practices

The two major life phases of a cannabis plant are vegetative growth and flowering or budding stage, both of which are controlled by light scheduling. High yielding, hardy and vigorous female plants are highly sought after for marijuana production. These traits are manipulated by genetic breeding and precise nutrient/ environmental management during the growth phases. Since genetics within the seed largely determine the quality of the final product, breeding for desirable traits is in huge demand and a commodity in itself. However, even with the best genetics plants can be poor producers if the environment is not managed in accordance to their physiological needs. Since cannabis requires high quantities of light, both plant spacing and light density can dramatically effect growth rates and, in turn, harvested yields (Vanhove 2011). A study in Belgium found that plants grown with 600 W lamps with 16 plants every $m^2$ yielded nearly double then the same varieties grown under 400 W lamps with 20 plants every $m^2$ (Vanhove, 2011). The going theory is that for every watt in a light fixture will result in 1 gram of final product per plant if other environmental conditions are managed properly. Hydroponic (soilless) production in indoor operations is becoming increasingly popular due to the level of control it gives the grower, though field production remains a popular choice for both hemp and marijuana producers.

## Propagation methods

All plants start as either a seed or as a clone, the propagation method used depends on the growers' preferences and overall system configuration. Clones are cuttings from a "mother" plant and ensure the same genetics, yields, and overall growth habits of the original plant. Another positive attribute is that the clones take less time then a seed to mature; this gives the grower a head start on production scheduling. An unlimited number of clones can be made from a single plant, generally during the vegetative stage for the best survival rates (Haze 2010). Cloning female plants with the most desirable characteristics ensures the same quality and predictable yields time after time, though it does limit the variety of strains that can be produced. When selecting for clones, it is recommended to cut 2-4" pieces off the tips of growing ends from branches toward the bottom of the plant (Haze 2010). This cutting is then dipped in a 500-1,500 ppm IBA

(Indolebutryic acid) rooting hormone solution, placed in a starting cube and left in a humid, warm environment (22-25 °C) until they are well rooted about a week (Cerveny et al. 2005). Cloning products that have positive reviews among growers include Clonex Gel, IBA Water Soluble Salts from Hortus, and homemade willow water (Anderson 2015). The willow water is of especial interest since it is all natural, inexpensive and naturally contains IBA and salicylic acid which helps both promote rooting and prevent infection from disease. Using a low-power CFL bulb with 16 hours of light and 8 hours of dark as supplemental lighting a day or two after clonal propagation helps to ensure the plants do not get burned during this critical time (Haze 2014). Light and humidity management are the top two concerns during clone establishment, misting the leaves with a light foliar spray containing Vitamin B-1 and/or Potassium silicate protectant could also enhance their growth (Haze 2010).

Seed:

Growing from seed is the most common propagation method in cannabis production, though it comes with an air of uncertainty. Ideally, seeds are feminized and their flowering periods are identified prior to starting but sometimes hermaphrodite or male seeds get in the mix. Feminized seeds are valuable because they are genetically ensured to produce only female plants, thus each seed being purchased could eventually produce buds whereas any hermaphrodite or male plants would be composted once their gender became apparent. That being said, when growing from seed (with the intention of harvesting buds) it is imperative to scan the plants as they grow for male traits and discard them prior to flowering. If one male/ hermaphroditic plant matures and pollinates the otherwise sterile females there will be seeds within the flower buds which is generally unacceptable for professional distribution- unless intentionally grown for seed or breeding purposes (Haze 2010). Cannabis plants are vigorous growers and can turn from a seed to a seedling with about 6 leaves in about a week. Seeds are first germinated by soaking in water (2-4 days) then transferred into growing medium after the first taproot breaks through the seed coat. After germination plants are either transferred to plug trays for vegetative growth or sowed directly into their final container.

It is recommended to avoid any soil mixes with added or extended release nutrients at this time since cannabis plants are very sensitive during their first few weeks of life. Utilizing organic seed starter mixes or other neutral soilless media starts helps avoid burning the new roots. Any additional fertilization should be done only after roots have established after the second week of growth (Haze 2010). Many growers have jumped on board organic and sustainable production techniques since medical grade product generally has more elite standards for quality and organic certification can demand higher prices. Building super soils that require little to no additional input besides environment and watering is on one end of the spectrum while fully controlled hydroponic systems with little to no soil storage capacity resides at the other. The cannabis production industry is very creative at maximizing space and light, there are countless methods documented online "grey" information forums. More research must be done on the subject in order to determine optimal closed-loop production systems since the majority of the information on this topic originates in forums of hazy legality and professionally.

Vegetative growth

Depending on the growers' preferences and the photoperiodism of the species, the plants are grown vegetatively until their desired size is reached or their light requirement is met, typically about 8 weeks for photoperiod plants and 6 for auto flowering ones (Haze 2010). During this stage cannabis grows rapidly and has high nutrient, light, and space requirements, but as long as the plants are getting enough (but not too much) of all three and the humidity and temperatures are right, they plants will be ready to flower in about 2.5 months (depending on the strain this could be considerably longer).

Flowering stage

When the plants reach target height/ volume the light schedule is switched and the flowering cycle begins for long-day obligate photoperiod plants. If they are autoflowering, as many *C. ruderalis* hybrids are, they will begin FBI without any additional manipulation around day 45 (Haze 2010). It is possible to keep some plants, generally cloning "mothers", in vegetative states for well over a year (Haze 2010). All plants take about an additional 40-60 days for buds to mature, though this will depend on climatic conditions as well as plant genetics (Haze 2010). It is important to supply higher levels of P and K and reduce the amount of N being fed to the plants to increase energy going to blooming flowers rather then vegetative growth. The changing color stigmas are the signal that the plants are ready to harvest. It is recommended to use a 30x magnifier to carefully inspect buds for the white hairs to turn a bronze color, when about 80% have changed this signals the trichomes are at the appropriate maturity level and contain the optimal amounts of biochemical compounds.

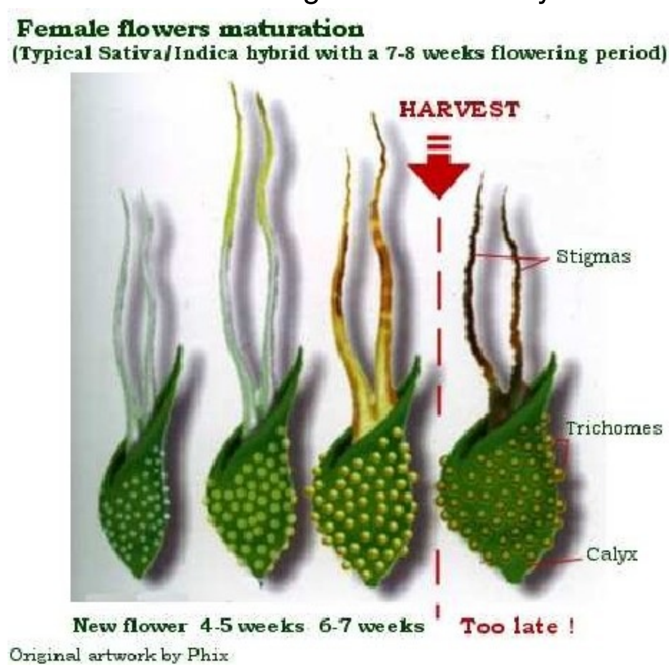


Figure 6. Diagram indicating the proper harvest time based on stigma maturation. (Vandermeer 2009).

Figure 7. Up close look at mature stigmas and the crystalline trichomes on a bud (Vandermeer 2009)

After trimming away all of the leaves and parts of the plant that are not the flower buds, the harvest is ready to be cured. This process involves drying out the buds in order to remove excess water and promote crystallization of cannabinoids. This can be done by hanging them in the open air and making sure they get adequate air flow to prevent molds and other unsavory characteristics. The curing process for marijuana is quite like that of wine, some terpenes and other aromatic qualities may express themselves only after long periods of time and their value may increase with these extra treatment times (Haze 2010). Shipping is generally as simple as vacuum-sealing the product in an industrial strength plastic bag, storage in plastic/ glass jars or packed in boxes, as long as the buds are not exposed to moisture and aren't getting completely crushed it is possible to be creative in this post-harvest task (Haze 2010).

A major issue in terms of sustainability of indoor cannabis production is the high intensity energy requirements of processes that control environmental conditions during growth. A research group on Energy Policy estimated that indoor cannabis growth accounts for 1% of the United States national electricity use, which translates to about $6 billion each year (Mills 2012). Additionally, on average one kg of final product equates to about 4600 kg of carbon dioxide emissions to the atmosphere; when all US production is added together it equals equivalent of about 3 million automobiles in terms of carbon output (Mills 2012). The immense carbon output of indoor plant production is an issue on multiple levels, especially in terms of long-term sustainability and efficiency. Breeding for resistant, autoflowering cultivars that can tolerate extreme weather conditions, combined with specific cannabinoid expression based on intended use can move production back out to the field were fewer energy intensive inputs are required. Additionally, modifying cultural methodology to shift towards more sustainable practices such as alternative energy sources, renewable resources (compost instead of synthetic fertilizers etc.) and streamlining production could also reduce waste and energy use.

**Production timeline:**

<u>Seedling: emerge in 1-5 days</u>

Week 1 Sow seed plug tray (200), place in mist house/ keep room humid and warm (80 RH% and temp) in Purple Cow Organic seed start or other soilless media such as coir, perlite, or vermiculite to promote air flow to the roots and adequate drying.

Vegetative stage: 2 weeks- 2+ months (depends on growers operation)

Week 2- germination, change light schedule. (LED lights for 18+ hours)

- Transplant to small containers or final pot size (2-3 gallon pot) in appropriate media mix - option to integrate extended release fertilizer or use hydroponics system. Use pH meter to test irrigation water and soil samples ensure proper levels every week (5.3-6.3 pH range) (ANLA 2004). There are many ways to deliver appropriate fertilization rates, the best thing to keep in mind is that during the vegetative stage Cannabis plants require high levels of nitrogen, anything from a 20-20-20 mix to specific 9-3-6 or extended releases can do the trick as long as all 16 micronutrients are obtained.

Week 4-6 - Transplant to final pot size- a minimum size of 2 gallon fabric pots can be used to to air prune roots (ANLA 2004). The volume of pot depends on the final size of the plant, root circling can be a disastrous problem with cannabis so it is important to plan out how large the plants will end up before transplanting to the final pot size.

Note: During vegetative growth a number of growth control techniques can be used to maximize lighting and final yields. These methods include: Low Stress Training (LST), Supercropping (Extreme LST), Screen of Green (ScrOG), topping, pinching (FIMing), Mainlinging/ manifolding, and defoliation (Haze 2010). All of these methods aim to control canopy height and maximize light and energy distribution to the colas- or flowering stalks. A short, even canopy with numerous colas will produce much larger yields in smaller spaces then an untrained plant would in the same environment (Haze 2010). In-depth information about these training techniques can be found online, an especially valuable resource is growweedeasy.com, this website is an accumulation of grower experiences and techniques laid out in an easy to follow guide for everyone from the most inexperienced to expert growers.

Flowering stage: 6 weeks- 4 months (depends on strain)

Week 8- FBI flower buds emerge with change of light- begin decreasing light hours increments of 2 hours per week until 12:12 is reached. Calcium and magnesium micronutrient should be applied to boost bloom, fertilizer NPK shifted to low N, high PK.

Week 14+- Buds should are harvested when 80% of the trichomes turn from white to bronze, the use of a 30x magnifying glass is recommended for this purpose.

Post- Harvest: 2.5 weeks- 1.5+ months

Drying: 3-10 days, slow drying results in best product. aim for 60% relative humidity and temperatures between 15.5-21 degrees C (Cervantes 2015).

Curing: 2 weeks- 1+ months, may be cured up to 6 months to increase potency.

Average total time to grow and cure cannabis: 3-4 months (though it can be more then 7 depending on strain requirements and length of curing process).

Figure 8. Buds undergoing the curing process in wide-mouthed mason jars (Harpha 2015).

Figure 9. Freshly harvested buds hang upside down to slow dry on organized lines (Harpha 2015).

## B. Current Production Statistics.

In 2014, the legal cannabis industry was valued at $2.7 billion dollars and is expected to continue to increase over the next five years (Dayton 2015). It is difficult to find accurate numbers that reflect cannabis production since it has been illegal for so long, much of the industry has gone “behind the scenes”. Countries are not very forthcoming with their own production rates, let alone value of the crop in the international market. However, the commodities produced from the cannabis plant fall into a wide range of categories including: medicinal extracts, dry herb, pills, edibles, beauty care products- topical applications, even seed genetics and growth systems and nutrient inputs have all become very specialized in catering to this market.

Afghanistan is currently in lead of global hashish production with an estimated 10,000-24,000 ha of plants grown annually, while this is not the most acreage, they are able to gain the largest yields (145kg/ha) out of nearly any country (UNODC 2015).

It is very difficult to pin down the amount of land and number of commercial growers at this time, since the whole system depends on a state-by-state basis of regulation within countries. On the whole global scale, production and consumption has continued to increase across the board (UNODC 2015). For example, Canadian hemp production was up to 27,000 ha in 2013 (Sawler et al. 2015). In the US most of the cultivation occurs in California, which accounts for over half of the production nationwide, close behind are Colorado, Oregon and Washington (Dayton 2015).

In July 2014, Governor Mark Dayton legalized the production of cannabis for therapeutic applications in Minnesota, and the selected producers intend to begin distribution to over 5,000 patients in 2016 (Nelson et al 2014). As with many newly legalized states, the government selects only the top performers in the greenhouse industry to produce the federally regulated substance. Currently Leafline Labs and Minnesota Medical Solutions are the only registered producers in the state of Minnesota that can legally produce and distribute cannabis products for the medical industry (Nelson et al. 2014). Leafline Labs consists of 10 members of the Bachman family business and will draw from their expertise in commercialized production, although the marijuana operation is to be separate from the garden chain. The state has some of the strictest laws around the consumption of the product; since smoking is prohibited it must be manufactured in either a pill or oil form. This plant lends itself well to controlled, even mechanized production strategies at the beginning, but it takes a human touch and understanding to bring the plant to full expression and maximum harvest ability months later. New production techniques, processing methods and specialized products are constantly on the rise and can be accessed via online forums where growers share their experimental results.

Cultivars and Series on the Market

| TABLE 1. Major *Cannabis sativa, indica* cultivars currently on the market and their indoor flowering times, Cannabinoid percents, and growth size (height x width). | | | |
|---|---|---|---|
| Plant Cultivars on the Market | Indoor Flowering time | Effects | Growth Size |
| Charlotte's Web (Sativa) | 8-9 weeks | 1% THC,<br>13-20% CBD | |
| Sour Diesel (70% sativa, 30% Indica) | 9-10 weeks | 20% THC | < 200 cm |
| Purple Kush (pure Indica) | 8 weeks | 22% THC | 60-90 cm |
| Avidekel (Sativa, Indica) | 10-11 weeks | 0% THC, 16% CBD | 130 cm |
| Auto Berry (Sativa/Indica/Ruderalis) | 6-7 weeks | 12% THC | 30-60cm |

Interest in high CBD, low THC varieties for medicinal applications is on the rise since these cultivars give therapeutic relief (especially anti-inflammatory) without the mind-altering effects. Charlotte's Web is one of the first successful breeding projects in this category has reached levels of only 1% THC and 13-20% CDB (GM 2015). However, these percentages are not consistent as the seeds will produce high-CBD plants between a 1:1 and 4:1 ratio of CBD:THC (OSBA 2015). This is problematic for growers striving for uniform products since only 25% of their plants will result in high CBD and low THC phenotypes, 25% will have high THC and low CBD, and 50% will express both high CBD and THC levels. In terms of quality control there is room for improvement of the genetics to create seeds that will continuously produce the precise cannabinoid levels desired, until that time high CBD strains should be grown only by cloning to ensure identical medical grade levels of bioactive compounds. Currently there are hundreds of strains on the market, each with its' own distinct flavors and bioactive terpene ratios.

Most varieties grown are a mix of sativa and indica, popular strains include: Sour Diesel, Purple Kush, OG Kush, Blue Dream and Auto Berry (GM 2015). Autoflowering is a valuable quality since it significantly reduces time to harvest, simplifies the growing process and can increase potency of the final product. Dinafem is a breeding company that specializes in crossing popular cultivars with *ruderalis* species to induce auto flowering into the genetics. Strains such as Auto Berry and a Sour Diesel x Haze 2.0 cross are examples of successful hybridization that enable fast turnover and smaller plants that are no longer light dependent. This type of production significantly reduces the amount of energy used- grow times can be shortened anywhere between 3-6 weeks depending on the species (GM 2015). Genetic breeding that selects for auto flowering also increases the hardiness and resistance of the plants to environmental stressors such as cold and water, two qualities that are useful especially in regards to outdoor production.


Figure 10. Auto flowering strain Dutch Passion AutoMazer produced 900g using a 1000W light, an unusually large harvest obtained by 'The King' using Heavy 16 fertilization system. (DP 2014)

When choosing a cultivar it is important to consider total grow time, final size of the plant, its photoperiodism, the size and consistency of flower buds, density of trichomes and aromatic qualities. All of these considerations will help determine the investment of time, energy and space needed for the operation, as well as the quality of the final product.

## IV. PROPOSED CROP TRANSFORMATION

### A. Crop Production Changes for the Future.

As the industry moves toward “greener” and low input production, pressure to reduce energy inputs and overall emissions will increase dramatically- especially with energy intensive crops such as cannabis. Additionally, indoor production may not be viable if legalization causes a price drop in final product as supply becomes more widespread. If operating costs are too high and growers are no longer obtaining the prices they relied on, many operations could become obsolete unless they can find more efficient methods to produce. With these limitations in mind, there are two recommended routes I envision that could increase the sustainability of the cannabis industry. The first involves breeding for plants that are hardy enough to grow outside with minimal inputs. These plants would be shorter and have large extensive root systems which allows them to find water in dry areas and resist extreme winds, increased disease and pest resistance and photoperiods that match the environments’ natural light cycles. In addition, the selection of cannabinoid expression could be customized based on the variety but focusing on the physical growth or “vehicle” that produces the flowers by breeding for resistance and hardiness could eliminate the need for high energy input indoor operations as a whole. Secondly, the methodology of cultivation on the technological side could be altered to promote energy efficiency. This would include selecting for auto flowering plants that have shorter life cycles (thus reduce energy consumption due to the time in cultivation), though this may just increase the number of plants grown in succession. Growers could also utilize a plethora of natural energy sources such as solar, geothermal, wind and closed loop methodology that fits with their specific operation. Tax breaks for those using less energy would help to promote sustainable practices, since they often cost more up front.

A New Production Schedule for *C. sativa, indica*

When it comes to formulating a “new” production schedule, it is difficult to determine what is truly a new idea since this industry is constantly evolving and experimenting on an individual basis. Across the field, and especially in regards to medical marijuana, there is a definite shift towards organic, sustainable production and way from synthetic inputs. An example of a direct comparison of production schedules can be seen in the attached chart, both are viable though one **utilized auto flowering varieties of the identified cultivar for a condensed timeframe.** Since this new schedule utilizes auto flowering varieties, more focus would be placed on **boosting soilless media fertility regimes by incorporating beneficial bacteria in a coir based mix and feeding with calculated applications of Heavy 16 liquid nutrient blends including: HEAVY Veg A, and HEAVY Bud A.** These are artisan grade formulas blended to ensure all micronutrient needs of the plant are met, about 1ml of solution is blended per gallon of water. Veg A is formulated for both hydroponics and soils and promotes vigorous foliar growth, while Bud A delivers the appropriate amounts of nutrients to slow down vegetative growth and promote the promotion of large, heavy buds. **Additionally, I would water with aerated compost tea to deliver micronutrients and beneficial populations of bacteria in one go.** The plants would continue to be grown in 3 gallon fabric pots to reduce the likelihood of circling roots, To top it off the auto flowering stains would **reduce production time anywhere from 3-6 weeks**, depending on strain. This is because plants **begin flowering after 4-6 weeks of growth, and can thus save the grower resources while producing considerable yields.** As for as final product goes, a significant portion would be **extracted to make concentrated oils and pills for therapeutic use, with less product sold as dry herb. This allows for specialty products such as medicinal salves to be created from the extracts and can target specific extract markets.**

### B. The New Crop Ideotype

The future of the cannabis industry is the continual development tightly controlled production operations that maximize yields and quality in a variety of ways. This applies not only to the methodology and cultural techniques used to raise the plants, which is enormously important, but also down to the genetic specifications expressed by the plants. Standardizing cannabinoid and other bioactive compounds in the buds is a major obstacle, especially in regards to high CBD, low THC strains such as Charlotte's Web and Avidekel. Since genetic stability has not yet been obtained, these strains are locked into clonal production methods when it comes to commercial production. Further breeding efforts to create phenotypes with repeatable genetic expression in regards to the high-CBD medical demand would help producers increase their quality and overall efficiency in their operation. Another breeding area of interest is the infusion of *ruderalis* auto flowering qualities into popular strains to reduce production time and in turn overall inputs. Plants that will flower independently of the lighting schedule opens up production to outdoor areas in the Northern hemisphere that may not receive the exact 12:12 ratio of sun to darkness, and eliminates the risk of ambient light ruining the FBI/ FBD of a sensitive photoperiodic strain. Additionally, *ruderalis* genes can be used to increase the cold and water hardiness, and decrease the growing size of a variety of cultivars which can be especially useful in regards to tropical *sativa* strains that may otherwise be intolerant of cold/smaller growing spaces. If all this were condensed into one plant, the future of the cannabis industry would have genetically reliable auto flowering phenotypes with specific ratios of cannabinoid expression for therapeutic applications.  Growers could produce a wide variety of smaller, hardy plants with properties to address a wide array of medical conditions in staggered stages of growth resulting in year round harvest opportunities. This would help maximize energy, water and space resources since higher turn over rates would allow for more production in less time.

## V. Acknowledgements

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Below is my Crop Production Schedule laid out in weeks with the two different methods, one with photoperiodic plants and the other with an auto flowering alternative.

| Crop<br>Scientific and/or Common Name | Series/Cultivar | Source | Seed or Veg. | Category: | Notes |
|---|---|---|---|---|---|
| *Cannabis sativa x indica* | Charlottes Web | OSBA (Old School Breeders Association) | seed | high CBD flower | covered |
| | | | | | |
| *Cannabis sativa x indica* | Charlottes Web-bred with *ruderalis* to auto flower | OSBA (Old School Breeders Association) | seed | high CBD flower- auto | covered |

| | | | | |
|---|---|---|---|---|
| **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 5** |
| 10 seeds sown | Radicle emer-gence | Change lights 18 hrs+ | Fertilization started | |
| Kept in mist house: 80% RH | 85% germinat-ed | pH 5.3-6.3 re-tain range | NPK: 9-3-6 | Sex plants-dispose of males/herms |
| 10 seeds sown | Radicle emer-gence | Change lights 18 hrs + | **Transplant to living soil. Veg A** | **Heavy 16 Veg A. Compost tea** |
| **Week 6** | **Week 7** | **Week 8** | **Week 9** | **Week 10** |
| | | | Switch light 12:12FBI Be-gins- | Switch fertilizer |
| | | | | NPK:10-30-20 |
| **Autoflower-FBI begins. H 16- Bud A** | **Aerated com-post /Cal/Mag** | **Bud A** | **Bud A** | **Bud A** |

| Week 11 | Week 12 | Week 13 | Week 14 | Week 15 |
|---|---|---|---|---|
| FBD:Apply mi-cronutrient | | First brown hair scouting | Begin flushing | |
| Cal/ Mag. | | Tricome matu-ration | Water only | |
| **Target Harvest** | **Curing Begins- trim/ hang slow dry** | **Buds go in Ma-son Jars: 60% RH** | **Curing- open jars daily** | **Curing- open jars daily** |

| Week 16 | Week 17 | Week 18 | Week 19 | Week 20 |
|---|---|---|---|---|
| | Target Harvest | Curing Begins | Buds go in Ma-son Jars | Continue curing process |
| | 80% of Tri-chomes are brown | Cut/ trim, hang upside down- slow dry | Open jars daily: 60% RH. 1-3 weeks | <6 months in-creases potency |
| **Curing- open jars daily** | **Curing- open 1x week** | | | |

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